

## Identification and characterization of factors involved in delayed effects of radiation

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Evidence from *in vitro* tissue culture studies, *in vivo* animal models, irradiated human subjects and radiotherapy patients, indicated that a variety of biological effects occur outside the radiation field and to the progeny of irradiated cells including genomic instability. The goal of this project is to develop an understanding of the molecular mechanism responsible for these responses. The unstable cell lines that we are using were isolated by Morgan and coworkers and are characterized by persistently elevated levels of oxidative stress and DNA damage, a condition that normally leads to programmed cell death but appears to drive continual chromosomal rearrangements. Morgan's group demonstrated that medium conditioned by unstable cell lines (RT, FE or LS-12) is extremely cytotoxic to parental GM10155 cells and was described as the death inducing effect (DIE). Thus, we hypothesized that the unstable cells release (through protein shedding and/or secretion) one or more factors into the media that causes stable cells to die primarily through apoptotic processes.

Our initial work began with biochemical approaches to determine the nature of the factor(s) responsible for DIE activity. For this we are using an iterative approach in that biochemical manipulated media is coupled with DIE activity. For these studies we use media conditioned by the growth of unstable cell lines collected over 48 hrs. Our results suggest that the DIE activity can be diluted 2-4 fold without loss of activity. Dialysis using 2- and 10-KDa pore sizes retained DIE activity indicating that the factor(s) are large molecules and unable to escape through the pores. When DIE media was treated with proteases, DIE activity was reduced. Similarly DIE activity was unaffected by temperatures as low as -20°C; however, activity was mostly lost when media was boiled. While not yet completed, these biochemical studies are consistent with DIE factors being proteins and likely derived from either shedding or secretory processes.

Since the DIE activity appears to be caused by protein(s) we are implementing mass spectrometry based proteomics approaches that will allow us to identify proteins in DIE medium and determine whether they are up- or down-regulated relative to controls. For this we are using mixed effects statistical models of mass spectrometry abundances fit with restricted maximum likelihood estimation and protein prophet to estimate relative concentrations and determine the number and uniqueness of peptides used for abundance estimates. In addition, the bioinformatics software by GeneGo called MetaCore will be used to infer the biological processes, pathway and networks altered by DIE activity and to guide DIE reconstitution experiment to confirm DIE factor identify.

Table 1. Biochemical and cellular characterization of DIE media: These preliminary results suggest that the factor(s) responsible for DIE activity are protein(s) and are produced by shedding or secretory processes. ND indicates not yet determined,

	Unstable cell-lines		
	<u>RT210B</u>	<u>Fe-10-3</u>	<u>LS-12</u>
Dilution factors	1-fold	>4-fold	1-fold
Dialysis			
2-KDa	Retained	Retained	Retained
10-KDa	Retained	Retained	Retained
Sensitivity to temperature			
-20°C	no	no	ND
100°C	yes	yes	ND
Sensitivity to proteolysis			
Trypsin	ND	yes	ND
Carboxypeptidase	ND	yes	ND
Proteinase K	ND	??	ND
Binding to lectin	??	??	??
Inhibition of shedding	No effect	ND	ND
Inhibition of secretion	ND	ND	ND

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